SUPPLEMENTARY MATERIALS AND METHODS

Case histories of patients with BRAF fusions.

Patient 1 (PAPSS1-BRAF): A 2.2 cm thick (Clark Level V), focally ulcerated, malignant melanoma from the left shoulder of a 27-year-old Caucasian female was screened for common melanoma driver mutations by the Vanderbilt melanoma SNaPshot assay (1) and determined to be "pan-negative". Axillary dissection revealed metastatic involvement of 21 of 25 axillary lymph nodes (initial tumor staging: pT4bN3 = Stage IIIC), and the area was subsequently irradiated. Unfortunately, she developed local recurrence and lung metastasis. She was subsequently treated with experimental and standard immunotherapies. The patient's disease progressed rapidly and she died nine months later (11 months after initial diagnosis). During the course of treatment, the primary shoulder lesion was sent to Foundation Medicine (Cambridge, MA) for comprehensive genomic profiling with the FoundationOne™ assay (2). The patient was consented on VICCMEL09109.

Patient 2 (TRIM24-BRAF): A 60-year old female with a history of breast cancer presented with a 4 mm-thick (Clark level III), ulcerated, primary malignant melanoma located over her right scapula. Immunohistochemical studies with appropriate controls demonstrated tumor expression of S-100 and Melan-A, supporting the diagnosis of melanoma and not breast cancer. The FoundationOne™ assay was performed on the primary tumor. The patient was consented in Israel; further clinical treatment data are not available.

FoundationOne™ Assay and Data Analysis. Formalin-fixed, paraffin-embedded (FFPE) blocks with a minimum 20% tumor content were sent to Foundation Medicine for the FoundationOne™ assay. DNA was extracted from 40μm of FFPE tissue using the Promega Maxwell 16 FFPE Plus LEV DNA Purification Kit according to manufacturer's instructions. DNA was quantified using a standardized PicoGreen fluorescence assay (Invitrogen). Molecular barcode-indexed ligation-based sequencing libraries were constructed using standard methods

(3) from at least 50ng and up to 200ng of sheared DNA depending upon how much DNA was available from a sample. Briefly, genomic DNA extracted from FFPE tissue sections was sheared to ~100-400bp by sonication before end-repair, dA addition and ligation of indexed, Illumina sequencing adaptors. Solution-based hybrid capture (3) with a custom Agilent SureSelect biotinylated RNA bait set was used to enrich all 3,320 exons of 182 cancer-related genes and 37 introns from 14 genes recurrently rearranged in cancer representing approximately 1.1 Mb of the human genome. The selected libraries were sequenced on an Illumina HiSeq 2000 platform using 49x49 paired-end reads.

Genomic DNA sequences were mapped to the reference human genome (hg19) using the Burrows-Wheeler Aligner (4) and processed using the publicly-available SAMtools (5), Picard, and Genome Analysis Toolkit (6). Genomic base substitutions were detected using a Bayesian methodology, which allows for the detection of novel somatic mutations at low mutant allele frequency (MAF) through the incorporation of tissue-specific prior expectations. The probability that a mutation is present in the data is evaluated using empirically observed error rates, and mutation candidates are issued if $p(mut / data)$>99%. Short insertions and deletions are detected by local assembly of data from each exon using the de-Bruijn approach, where candidate mutations with maximal number of supporting reads are retained. Mutations were filtered for strand bias (Fisher's test, p<1e^(-6)), and read location bias (KS test, p<1e^(-6)), the presence in 2 or more normal controls or verified in dbSNP v.135 (7), and subsequently annotated for known and likely somatic mutations using the Catalogue of Somatic Mutations in Cancer (COSMIC) (8). Copy number alterations were detected by comparing targeted genomic DNA sequence coverage at all exons with a process-matched normal control sample as well as allele frequencies of ~1,800 genome-wide SNPs, and fitting data to a model of absolute copy numbers and tumor purity. Focal amplifications are called at segments with ≥6 copies and homozygous deletions at 0 copies, in samples with purity >20%. Genomic rearrangements were detected by clustering chimeric reads mapped to targeted introns.

Targeted RNA Sequencing and Data Analysis. Total RNA was extracted from 40µm of FFPE tissue (same block used for DNA extraction) using a prototype kit for the Promega Maxwell according to the manufacturer's instructions and was quantified using a standardized RiboGreen fluorescence assay (Invitrogen). Total RNA was reverse transcribed with random hexamer primers by the SuperScript® III First-Strand Synthesis System (Invitrogen) to make cDNA. Double-stranded cDNA was made with the NEBNext® mRNA Second Strand Synthesis Module (New England Biolabs) (9) and used as input to library construction. Molecular barcode-indexed ligation-based sequencing libraries were constructed using standard methods (3) from double-stranded cDNA starting with end-repair and followed by dA addition and ligation of indexed, Illumina sequencing adaptors. Solution-based hybrid capture (3) with the Agilent SureSelect Kinome Kit was performed according to the protocol in Levin *et al*, 2009 (10) and the selected libraries were sequenced on an Illumina HiSeq 2000 platform using 49x49 paired-end reads.

cDNA sequences were mapped to the reference human genome (hg19) and transcriptome (RefSeq) using the Burrows-Wheeler Aligner (4) and processed using the publicly available SAMtools (5), Picard, and Genome Analysis Toolkit (6). Gene fusions were detected by clustering chimeric reads mapping to separate genes.

cDNA Constructs. The BRAF V600E FLAG-tagged plasmid was described previously (11). Using Herculase II Fusion Polymerase (Agilent), full-length wildtype (WT) *PAPSS1* was cloned from 293H cell line cDNA. To create the FLAG-tagged PAPSS1-BRAF construct, exons 1-5 of *PAPSS1* were cloned from 293H cell line cDNA, and exons 9-18 of WT *BRAF* were cloned from a WT BRAF FLAG-tagged construct. Primers capturing exon 5 of *PAPSS1* and exon 9 of *BRAF* contained *BRAF* and *PAPSS1* sequence, respectively, such that *PAPSS1* and *BRAF* could be "sewn" together through a subsequent PCR reaction. Similarly, exons 1-9 of *TRIM24* were cloned from 293H cell line DNA and "sewn" to exons 9-18 of WT BRAF(-FLAG). PCR products

were then cut with restriction enzymes and ligated into the pcDNA3.1+ vector (Invitrogen). C-terminal FLAG tags were added to the WT *PAPSS1* sequence by PCR. Direct sequencing of the WT *PAPSS1(-FLAG)*, *PAPSS1-BRAF(-FLAG)* and *TRIM24-BRAF(-FLAG)* constructs was performed to confirm the sequences and to ensure no other mutations were introduced during the cloning process. Primers are listed in Supplementary Table S2.

Transfections, Drug Treatment and Immunoblotting. 293H cells (Invitrogen) were transfected using Lipofectamine 2000 (Invitrogen). After 24hr (BRAF V600E, PAPSS1, PAPSS1-BRAF) or 48hr (TRIM24-BRAF), cells were serum-starved for 6hr then treated with vehicle (DMSO), PLX4032/vemurafenib (Chemietek), or GSK1120212/trametinib (Chemietek) for 2hr. Cells were then lysed using standard RIPA buffer supplemented with protease and phosphatase inhibitors. Lysates were quantified and subjected to SDS-PAGE and immunoblot analysis using antibodies against the following targets: phospho-MEK1/2 (Ser217/221), total-MEK1/2, phospho-ERK1/2 (Thr202/Tyr204), total-ERK1/2, BRAF and FLAG. All antibodies were purchased from Cell Signaling except for anti-BRAF (C-terminal, SantaCruz) and anti-FLAG (Sigma-Aldrich).

BRAF Fusion Detection and Reverse Phase Protein Array (RPPA) Analysis from TCGA Data. Raw RNA, whole genome, and whole exome sequencing files from TCGA (n=266, skin cutaneous melanoma dataset) were downloaded and raw reads spanning exon 7 (chr7:140500162-140500281) through exon 12 (chr7:140477791-140477875) of BRAF were extracted from indexed .bam files (SAMtools, (5)). Upon manual inspection, two potential BRAF fusions in SNaPshot “pan-negative” patients were identified (TAX1BP1-BRAF and CDC27-BRAF, **Figure S4**). Level 3 processed RPPA and mutation data were accessed via the Broad Institute TCGA Genome Data Analysis Center (2013), skin cutaneous melanoma (primary and

metastatic solid tumor cohort). Samples evaluated for both mutation and RPPA (N=200), were merged and plotted (beeswarm, R package) according to mutation status **(Figure S5)**.

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